Application No.: 09/980,468
Inventor: HEINZ et al.
Reply to Office Action of 27 January 2006
and the Advisory Action of 17 May 2006
Docket No.: 0093/00029

REMARKS/ARGUMENTS

Claims 1-5, 8-14, 16-20 and 22-29 are pending. Claims 1, 4-5, 8-12 and 24-29 are currently rejected. Claims 2-3, 13-14, 16-20 and 22-23 are withdrawn. Claims 1 and 8 have been amended to clarify grammatical issues. No new material has been added.

Rejection under 35 USC §112

Claims 1, 4-5, 8-12 and 24-29 stand rejected as allegedly not enabled.

Applicants wish to thank Examiner MCELWAIN for courtesies extended to their representative during a phone interview on or about 01 June 2006. The interview attempted to clarify issues relating to the Office Action of 27 January 2006 and the Advisory Action of 17 May 2006. The Examiner asserted that the specification does not make clear what sequences are used in what Examples. Applicants provide a brief explanation of the relevant Examples illustrating the sequence being utilized:

As set forth in Example 6, DNA sequences that code for Cer1, Cer3 and Cer16, which encode Δ6-acetylenase/desaturases, were isolated using known DNA sequences. Degenerate oligonucleotides were derived from Δ5 and Δ6 desaturases and by means of PCR with *Ceratodon purpureus* cDNA, the following fragments were obtained.

Cer1 = SEQ ID NO: 5

Cer3 = SEQ ID NO: 7

Cer16 = SEQ ID NO: 9

The corresponding proteins of Cer1 and Cer3 show similarity with a Δ6-acyl-lipid desaturase of *Physcomitrella patens* and Cer 16 shows similarity with Δ6-acyl-lipid desaturase and Δ8-sphingolipid desaturase from higher plants.

PCR amplification of a *Ceratodon purpureus* cDNA library with primers according to Cer1, Cer 3 and Cer16 showed that the library contains the Cer clones .

As set forth in Example 7, full length DNA clones from *Ceratodon purpureus* were obtained from further screening of the cDNA library with Cer1, Cer3 and Cer16. Full length clones of Cer1 and Cer3 were obtained which were subcloned in puc19 vectors and subsequently, *E. coli* were transformed with the vectors.

The two *E. coli* clones, designated Cer1-50 and Cer3-50, were sequenced and correspond to SEQ ID NO: 1 and SEQ ID NO: 11, respectively. The deduced protein sequence of each contains three histidine boxes at the C terminus which are representative of desaturases wherein the first histidine is exchanged for glutamine, another characteristic of Δ5- and Δ6-acyl-lipid desaturases and Δ8-sphingolipid desaturases.

As set forth in Example 8, complete functional clones of Δ6-acetylenase/desaturases were made and cloned into vectors for functional expression. The oligonucleotides for PCR to make the functional clones were initially derived from Cer1 and Cer3 cDNA with the primers derived from Cer1 adapted for expression in yeast. Δ6-acetylenase/desaturases cDNA from *Ceratodon purpureus* served as the PCR template. The resulting fragment was ligated into a vector and clones were identified by control cleavage pBS-Cer1.

The cDNA of the clone Cer50, a mono-functional Δ6-desaturase that corresponds to SEQ ID NO.: 3, may also be used in an analogous manner.

Functionality of the encoded enzyme was verified by transforming yeast with an expression vector ligated to the excised fragment derived above from the PCR reaction.

As set forth in Example 9, referring to table 1 (attached hereto), wherein the data shows that yeast mutants transformed with Cer generated fragments, as described above, had increased amounts of unsaturated fatty acids, i.e., an increase in acetylenase/desaturase activity. Cer and empty cassette transformed yeast were provided various fatty acids. The total fatty acid composition, in mol%, was determined providing an assessment of enzymatic activity.

Turning now to the results themselves, column headers of table I indicate the fatty acid added to that yeast culture and the Examiner is first directed to the column labeled α-18:3 (α-

linolenic acid, $18:3^{9,12,15}$). The results for the Cer transformed yeast, Cer1/pYES2 and Cer3/pYES2, show an increase in the mol% of $18:4^{6,9,12,15}$ (stearidonic acid), while the control empty cassette yeast, pYES2 had no $18:4^{6,9,12,15}$. The increase of $18:4^{6,9,12,15}$ in Cer1/pYES2 was from 0 to 1.7 mol% and in Cer3/pYES2 from 0 to 1.9 mol%. Thus, transformed yeast have enzymatic activity to convert $18:3^{9,12,15}$ to $18:4^{6,9,12,15}$ that is absent in empty cassette transformants.

Further, the Examiner is next directed to the column labeled 18:2 (α-linoleic acid, $18:2^{9,12}$). $18:2^{9,12}$ is converted to $18:3^{6,9,12}$ (γ-linolenic acid) and the yeast transformed with Cer1/pYES2 and Cer3/pYES2 show increases from 0 to 0.8 and from 0 to 8.1 mol%, respectively. No change in the mol% of $18:3^{6,9,12}$ in the control cassette can be seen in the data. These data provide additional evidence of the increased acetylenase/desaturase activity in the Cer mutants when compared to empty cassette transformed yeast.

These data provide additional evidence of the increased acetylenase/desaturase activity in the Cer mutants when compared to empty cassette transformed yeast. Yeast transformed with the Cer1 and Cer3 fragments have enzyme activity that is absent in the non-Cer transformed yeast.

In sum, the specification is clear to one of ordinary skill in the art and enables the claimed invention. The descriptions of the steps involved in producing the transformed yeast and the data in table 1 provide evidence for the functionality of the claimed isolated nucleic acid sequence that codes for a polypeptide having Δ6-acetylenase and/or Δ6-desaturase activity. Accordingly, Applicants respectfully request withdrawal of the enablement rejection.

Conclusion

Applicants respectfully submit that the present application is in condition for allowance, which action is courteously requested. Please charge any shortage in fees due in connection with the filing of this paper to Deposit Account 14.1437. Please credit any excess fees to such account.

Respectfully submitted,

Todd R. Samelman
Registration No.: 53,547

NOVAK DRUCE DELUCA & QUIGG, LLP
Customer No.: 26474
1300 Eye St. N.W.
400 East Tower
Washington, D.C. 20005
Phone: (202) 659-0100
Fax: (202) 659-0105

Attachments

Table 1

Fatty acids [mol %]	pYES2					Cer1/pYES2					Cer3/pYES2				
	-	18:2	γ-18:3	α-18:3	18:4	-	18:2	γ-18:3	α-18:3	18:4	-	18:2	γ-18:3	α-18:3	18:4
16:0	26.2	24.1	27.8	27.4	32.7	24.2	23.1	26.2	26.7	26.5	26.5	23.3	28.1	29.2	28.6
16:1^{9}	41.8	9.6	27.4	27.3	16.1	36.5	13.3	24.7	28.8	21.9	43.8	9.9	25.2	34.0	20.9
16:2^{9,6}						6.9	1.8	3.3	6.3	3.0	1.1		0.1	0.8	0.1
18:0	6.5	5.3	6.1	6.1	7.9	6.4	6.1	6.6	6.5	7.1	5.5	5.3	6.3	5.8	5.9
18:1^{9}	23.6	4.9	15.1	14.8	11.3	24.9	8.8	15.6	20.0	16.8	21.4	6.3	15.7	14.3	11.5
18:2^{9,6}						0.3		0.2	0.3	0.2	0.1			0.1	
18:2^{9,12}		53.9					41.9					42.3			
18:3^{9,6,12}			19.5				0.8	16.1				8.1	21.2		
18:3^{9,12,15}				22.8					10.0					11.9	
18:4^{9,6,12,15}					28.8				1.7	21.3				1.9	30.1
18:3^{9m,6,12}							1.3	4.6							
18:4^{9m,6,12,15}										2.3					
ΣDes. [mol %]	-	-	-	-	-	7.2	3.9	8.1	7.3	5.8	1.2	8.1	0.1	2.8	0.1